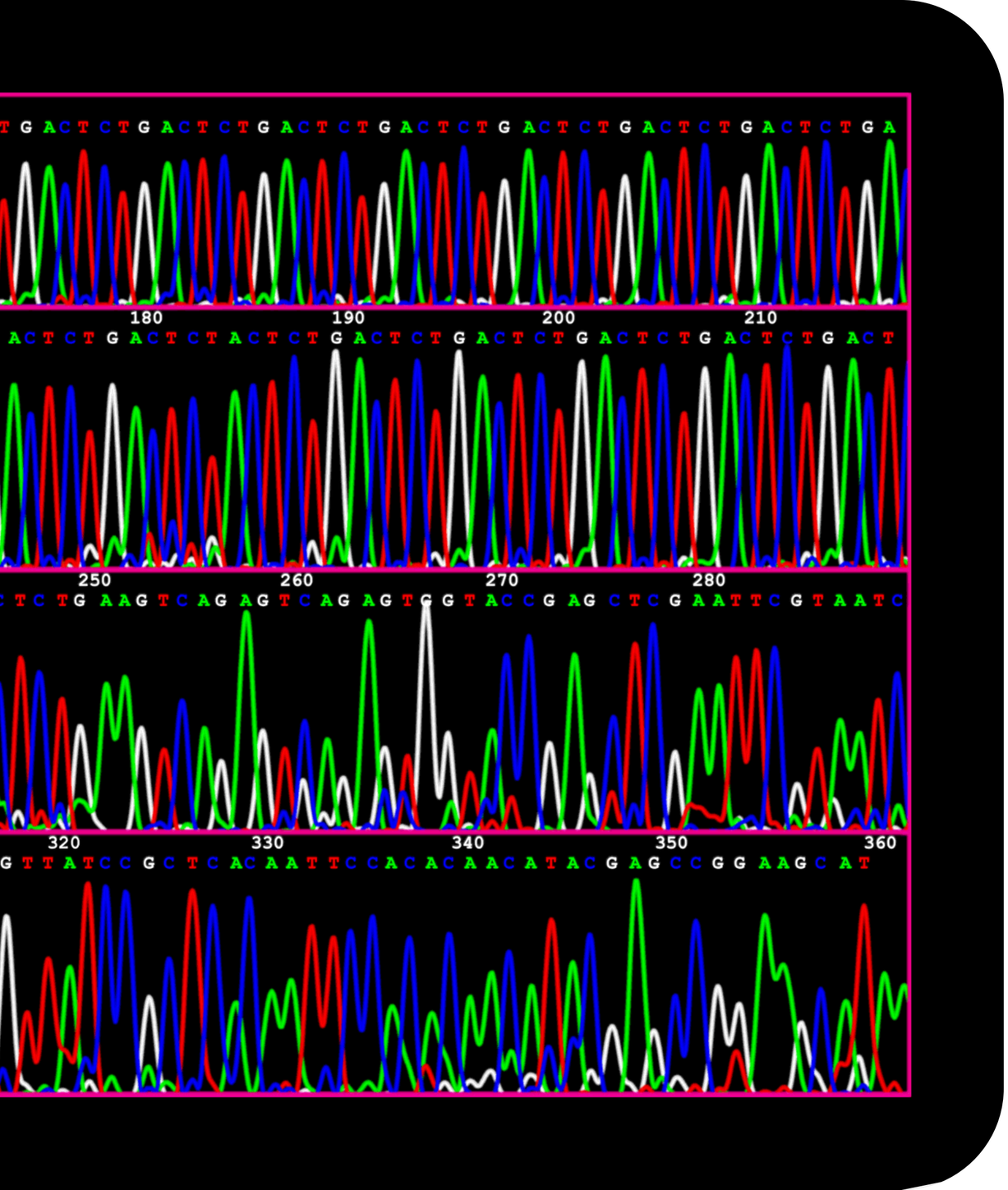
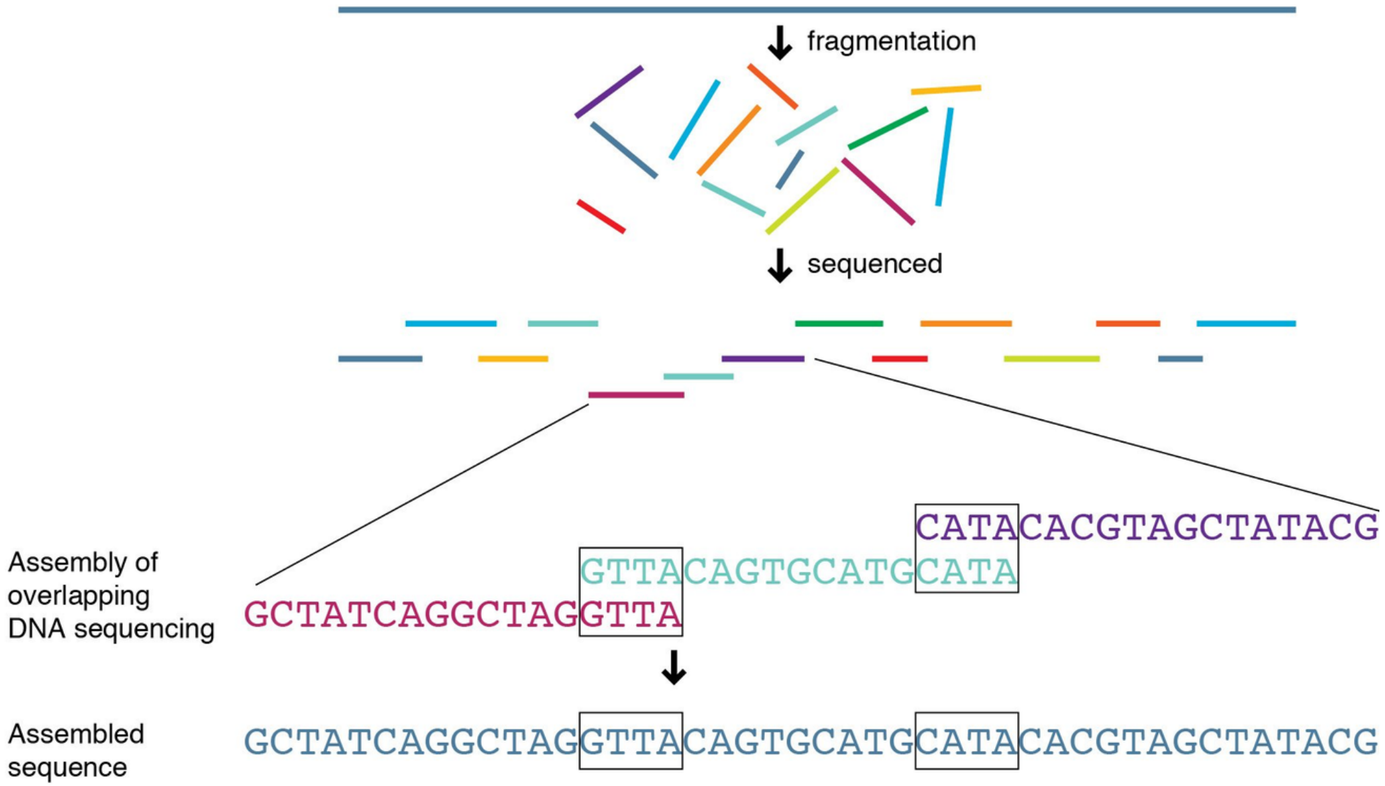


Sequencing Methods Overview

Exploring DNA Sequencing Techniques

- Swayam Roll no.114
- Mudassir Roll no.128
- Nayan Roll no.110

Introduction



- The process involves identifying the exact sequence of nucleotides (A, T, C, G) in a DNA molecule.
- This advancement has transformed our comprehension of life's basic components.
- It has become an essential asset in diverse areas such as genomics, molecular biology, medical diagnostics, and evolutionary research.

Sequencing Methods

01

Sequencing by Synthesis

Sequencing by synthesis involves the detection of the nucleotides as they are incorporated into a growing DNA strand.

02

Ion Semiconductor Sequencing

Ion semiconductor sequencing is a method of DNA sequencing that is based on the detection of hydrogen ions that are released during DNA synthesis.

03

Single Molecule Real-Time Sequencing (SMRT)

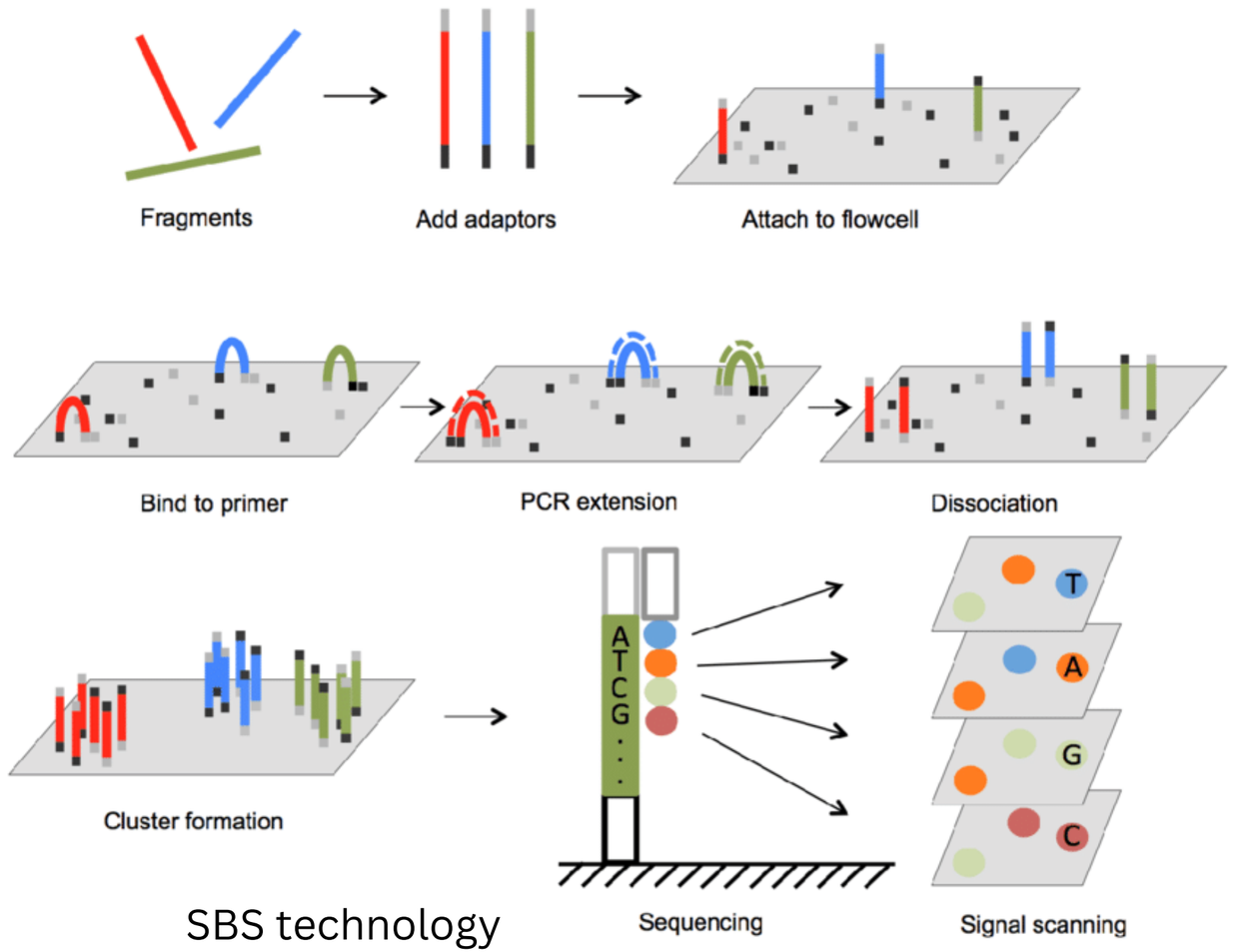
Single Molecule Real-Time Sequencing (SMRT) is a method of DNA sequencing that involves the detection of the release of light that occurs when nucleotides are incorporated into a growing DNA chain.

04

Nanopore Sequencing

Nanopore sequencing detects DNA bases by monitoring disruptions in electrical current as DNA strands pass through nanoscale protein pores.

Sequencing by Synthesis



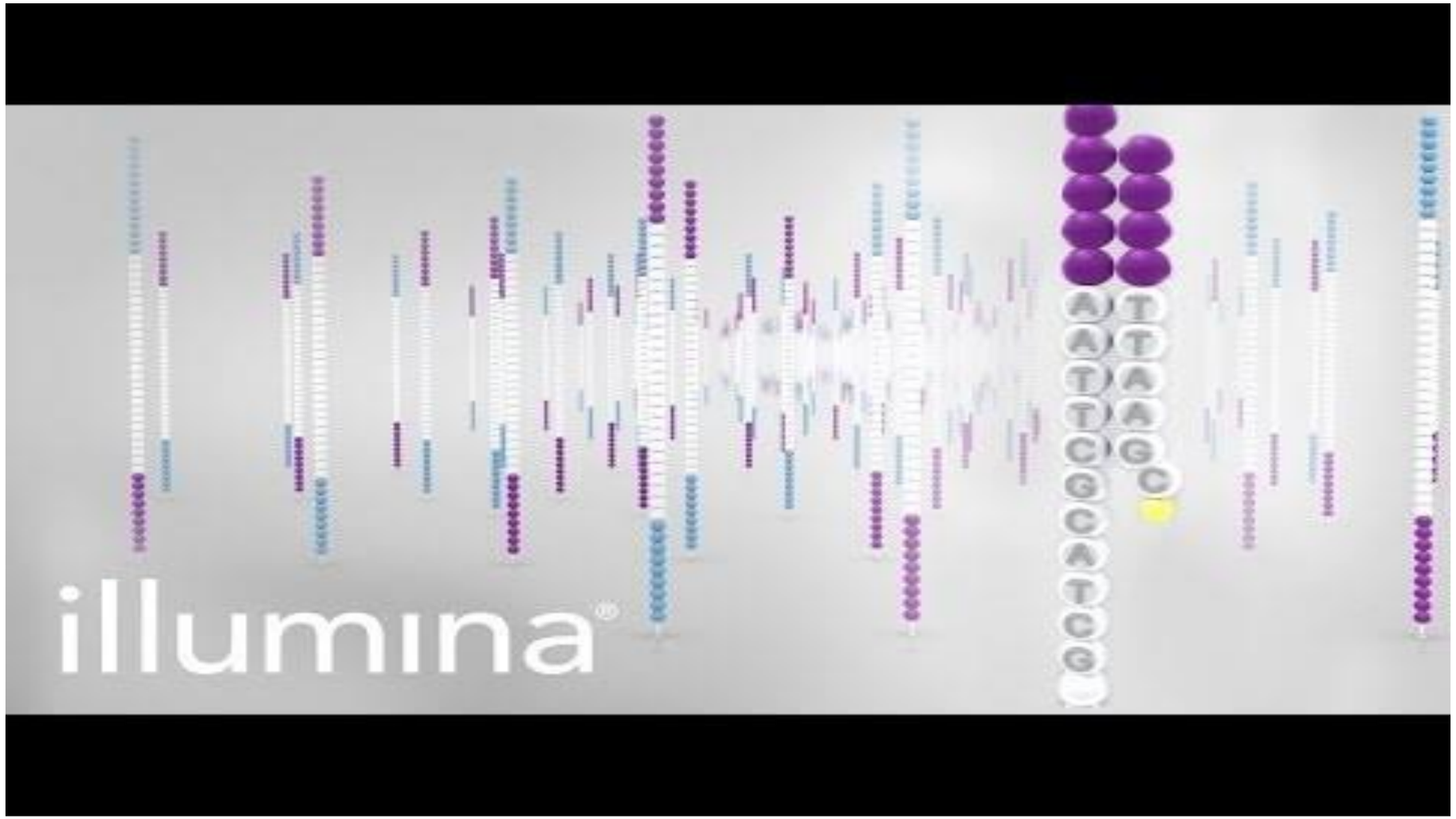
SBS technology

source: Researchgate

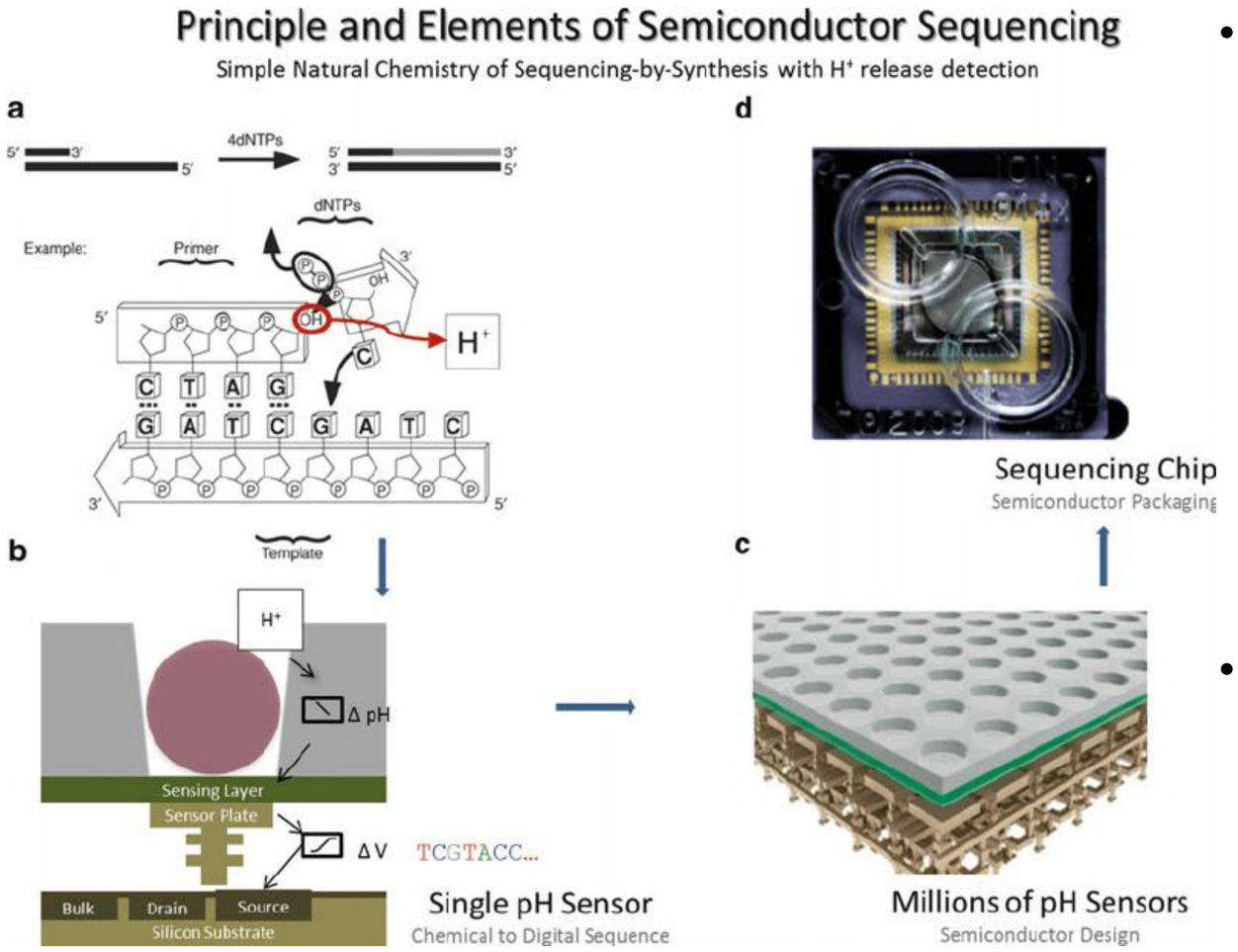
- Also known as Cycle Sequencing or Sequencing by Synthesis (SBS), is a widely used method for determining the order of nucleotides in a DNA molecule. This technique is employed by popular platforms such as Illumina and has become a cornerstone of modern genomics research.

- The principle behind Sequencing by Synthesis involves the use of fluorescently labeled nucleotides and a cyclical process of nucleotide incorporation, imaging, and cleavage.

Sequencing by Synthesis



Ion Semiconductor Sequencing

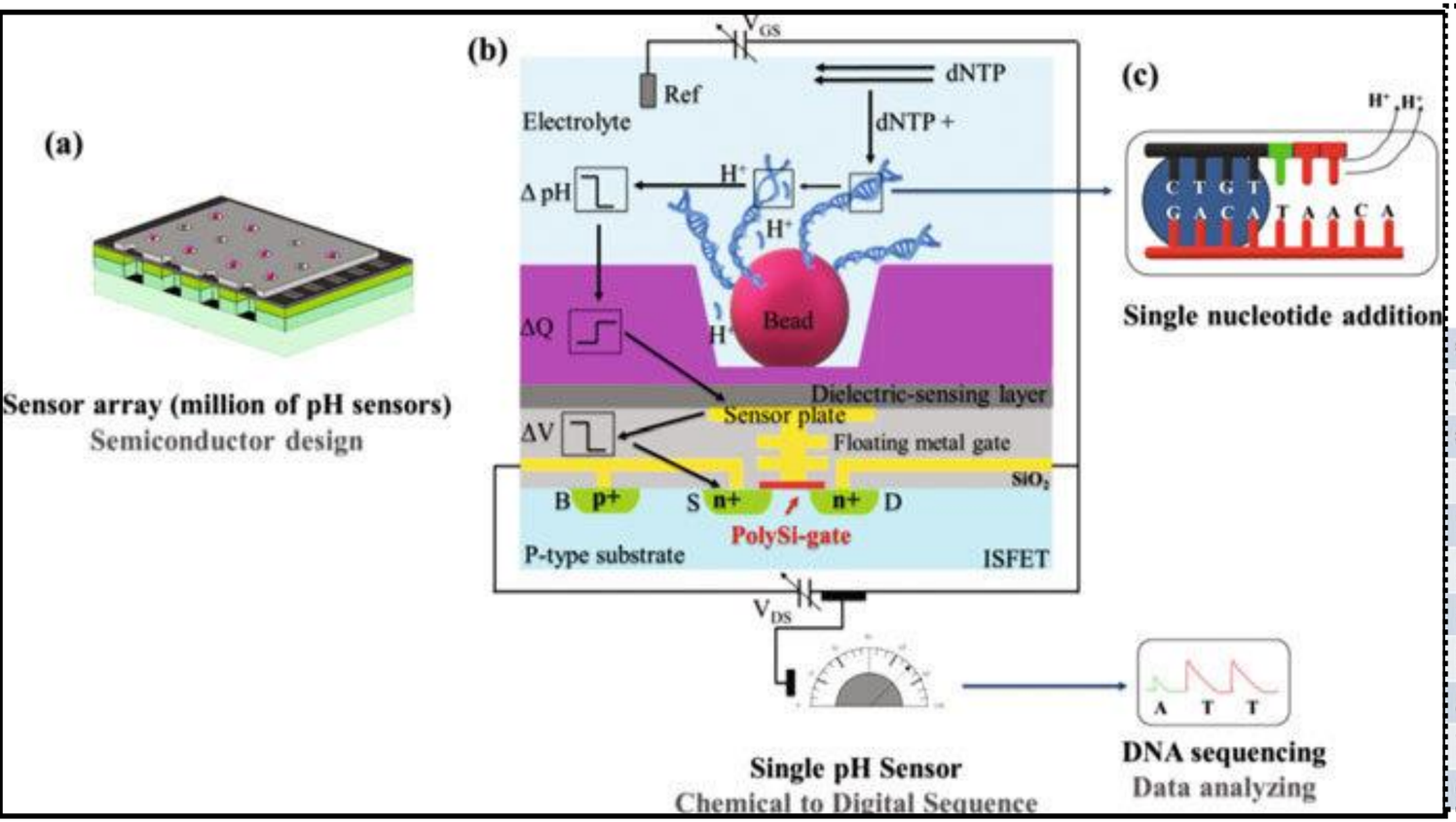


Ion Semiconductor Sequencing

source: Researchgate

- Also known as Ion Torrent Sequencing, is a unique method developed by Ion Torrent (now part of Thermo Fisher Scientific). This technology revolutionized DNA sequencing by eliminating the need for fluorescent labeling and optical detection, instead relying on the direct detection of hydrogen ions released during DNA synthesis.

- The principle behind Ion Semiconductor Sequencing is based on the detection of pH changes caused by the release of hydrogen ions (H+) during the incorporation of nucleotides into the growing DNA strand..

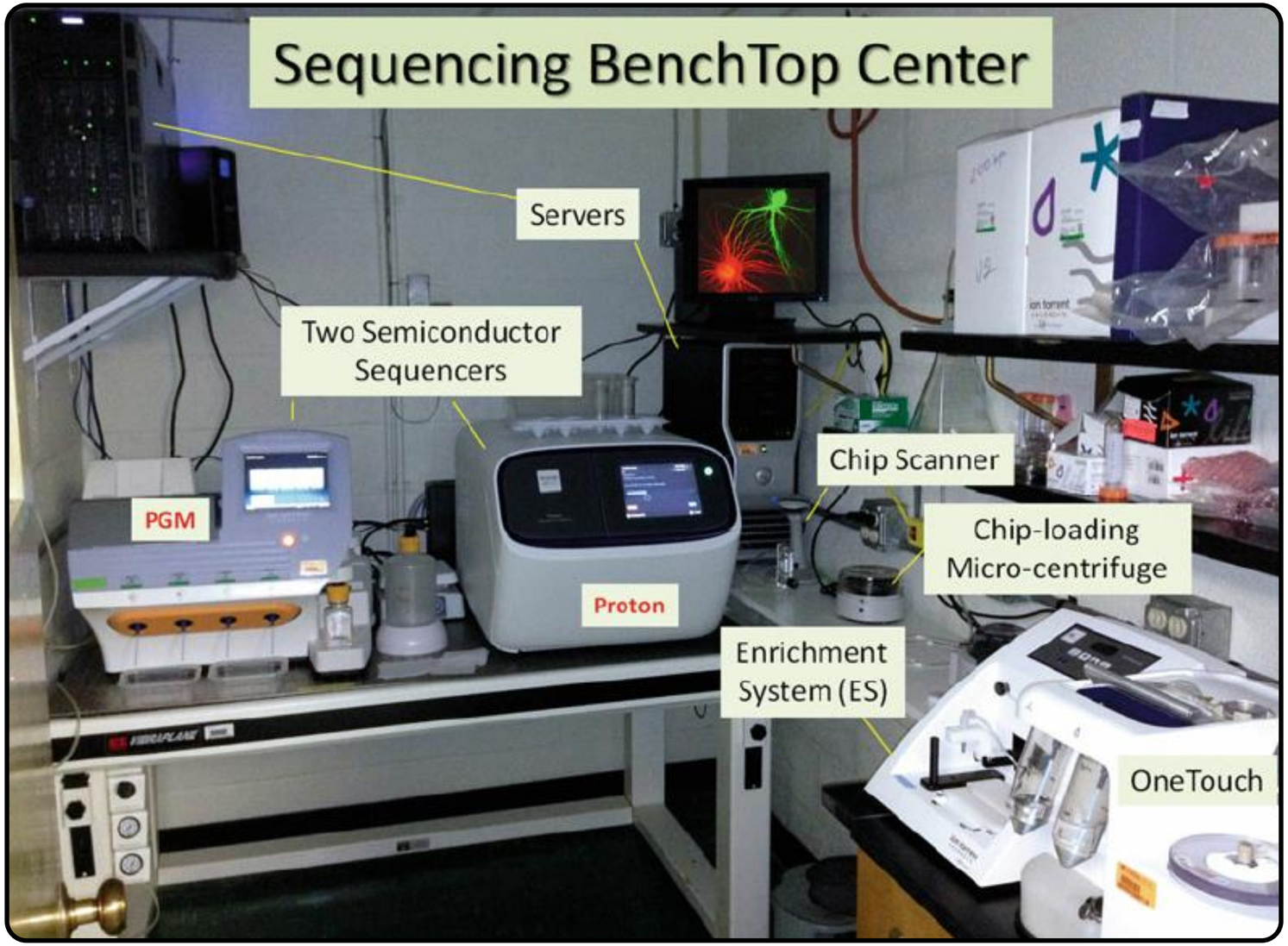


Semiconductor Sequencing Chips

Chip Types[1]	314	316	318	IP1/IP2/IP3*
# Wells per Chip	1,262,528	6,348,216	11,302,473	165 M/660M/1.2B
Volume, µL	7	30	30	55
# of Reads[1]	295,736	1,592,020	4,580,123	124-496,000,000
Yield/Q20, bases	24.6/**21.9** Mb	146.7/**122.5** Mb	600/**500** Mb	**10** / **60** / **480** Gb
Mean Read[1], bp	83	92	129	Up to 300
Longest Reads[1]	396	307	386	640
Run Time[1], Hrs	2.4	3.1	4.5	~4
Processing, Hrs[1]	0.3	2.0	4.5	Up to 8 hrs
Analysis[2], Hrs	12	18	30	Up to 1 day
Template Molecules	2.5×10^7	5×10^7	5×10^7	2.5×10^7
Cost per Run	$400	$500	$800	$1,000

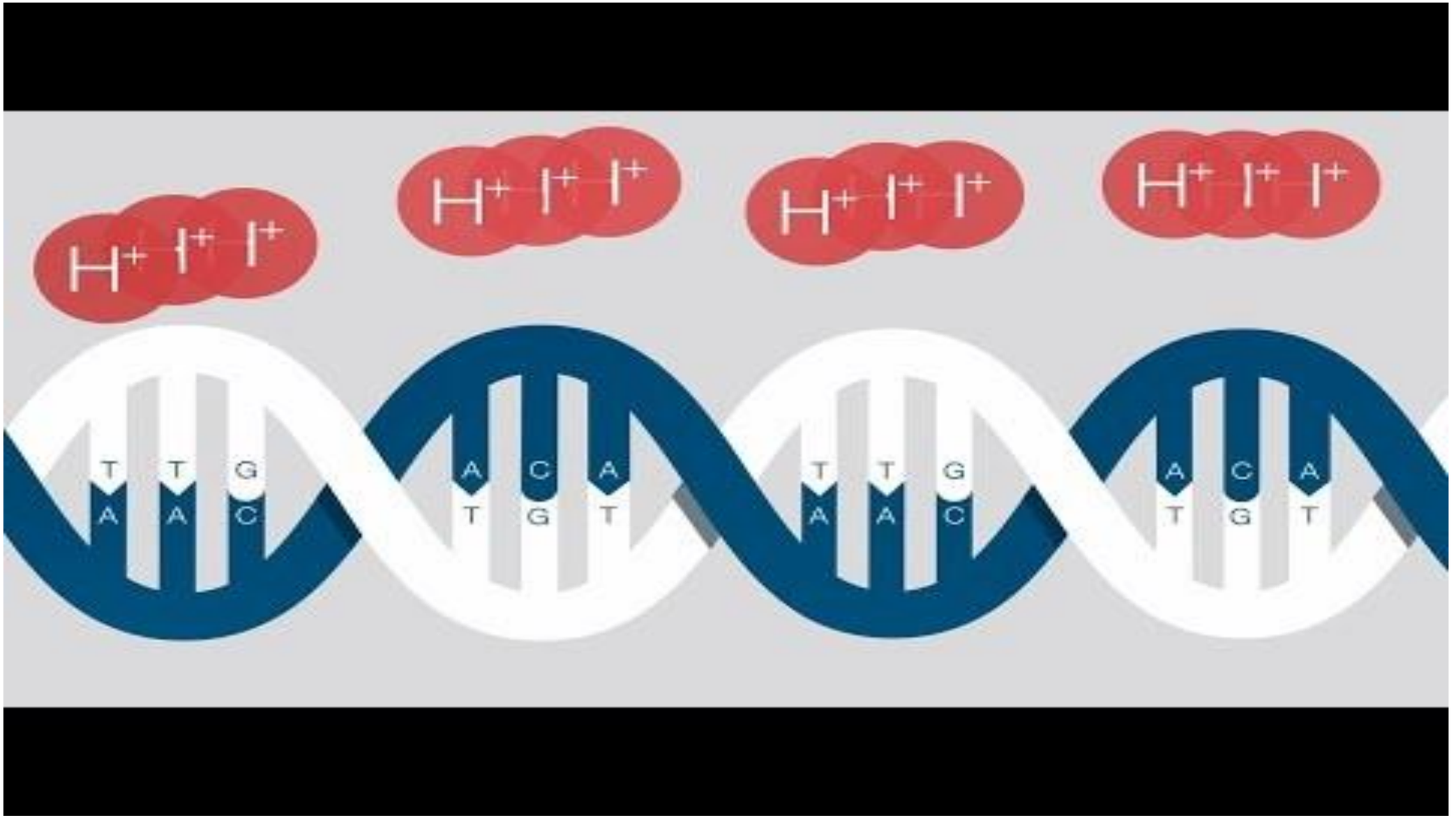
Ion Semiconductor Sequencing

source: Researchgate

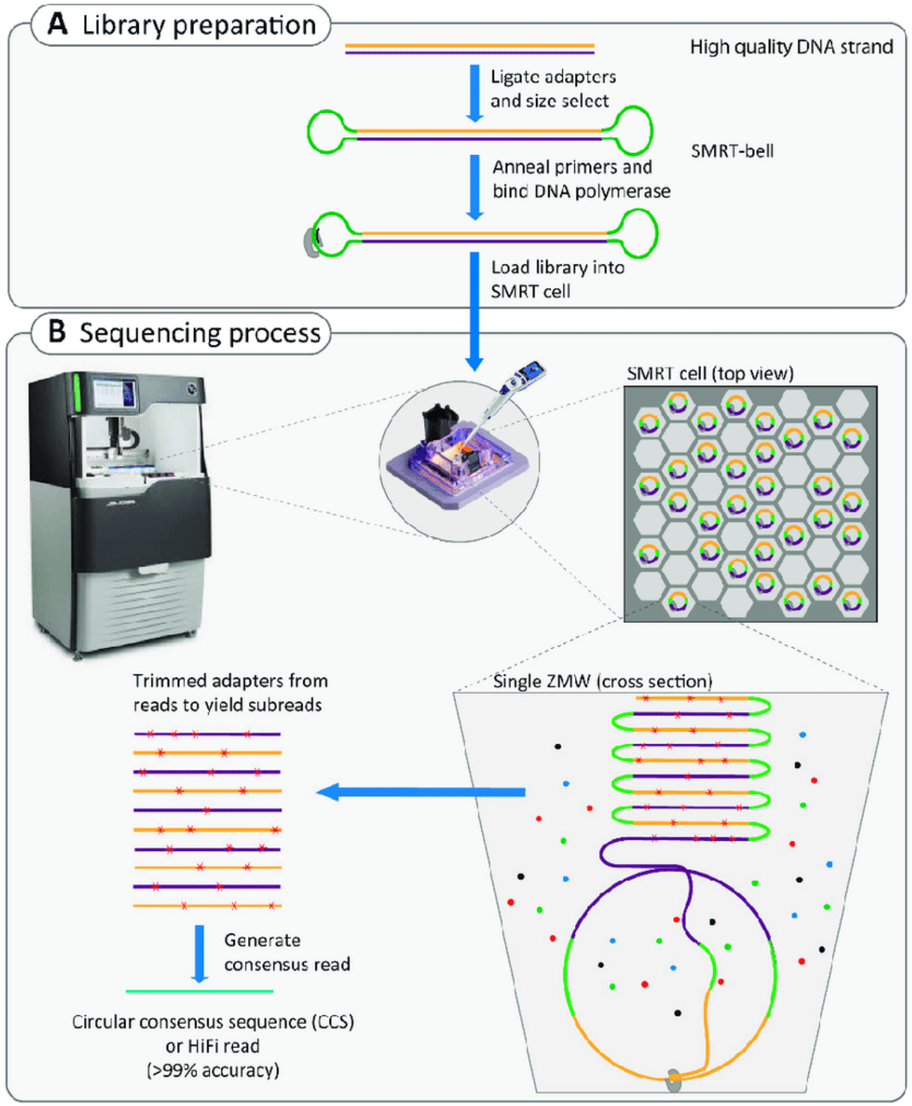


- Ion Semiconductor Sequencing offers fast run times, scalability, and the ability to sequence small to medium-sized genomes or targeted regions. However, it generally produces shorter read lengths compared to some other methods and can be more prone to errors in homopolymer regions (consecutive repeats of the same nucleotide).

- Despite these limitations, Ion Torrent Sequencing has found applications in various fields, including microbial genomics, targeted sequencing, and gene expression analysis, due to its speed, cost-effectiveness, and ease of use.

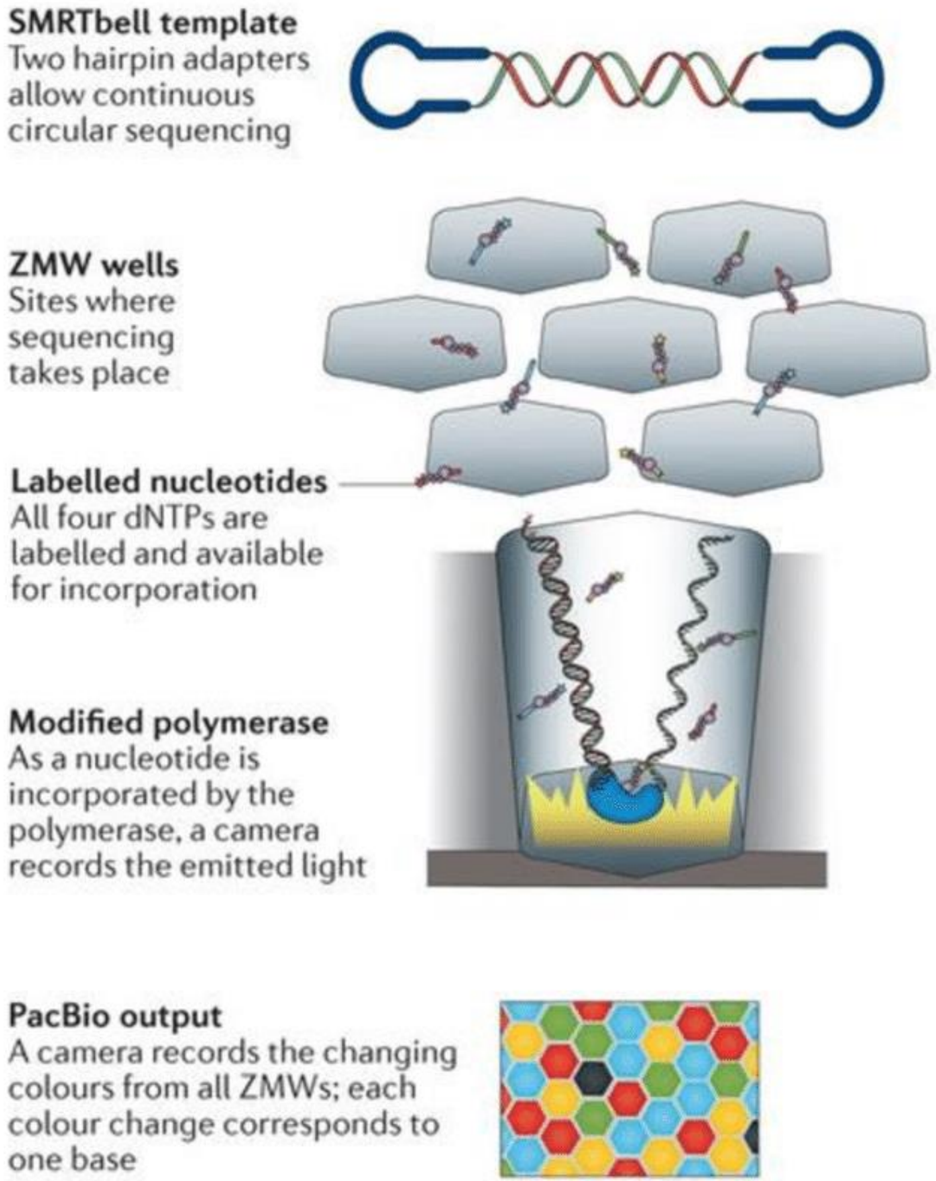

H+ I+ I+
H+ I+ I+
H+ I+ I+
H+ I+ I+
T T G
A A C
A C A
T G T
T T G
A A C
A C A
T G T

SMRT (Single-Molecule Real-Time) Sequencing



- SMRT Sequencing, developed by Pacific Biosciences, is a groundbreaking technology that revolutionized DNA sequencing by enabling the real-time observation of DNA synthesis at the single-molecule level. This method provides long read lengths and the ability to detect base modifications, making it a powerful tool for various applications.

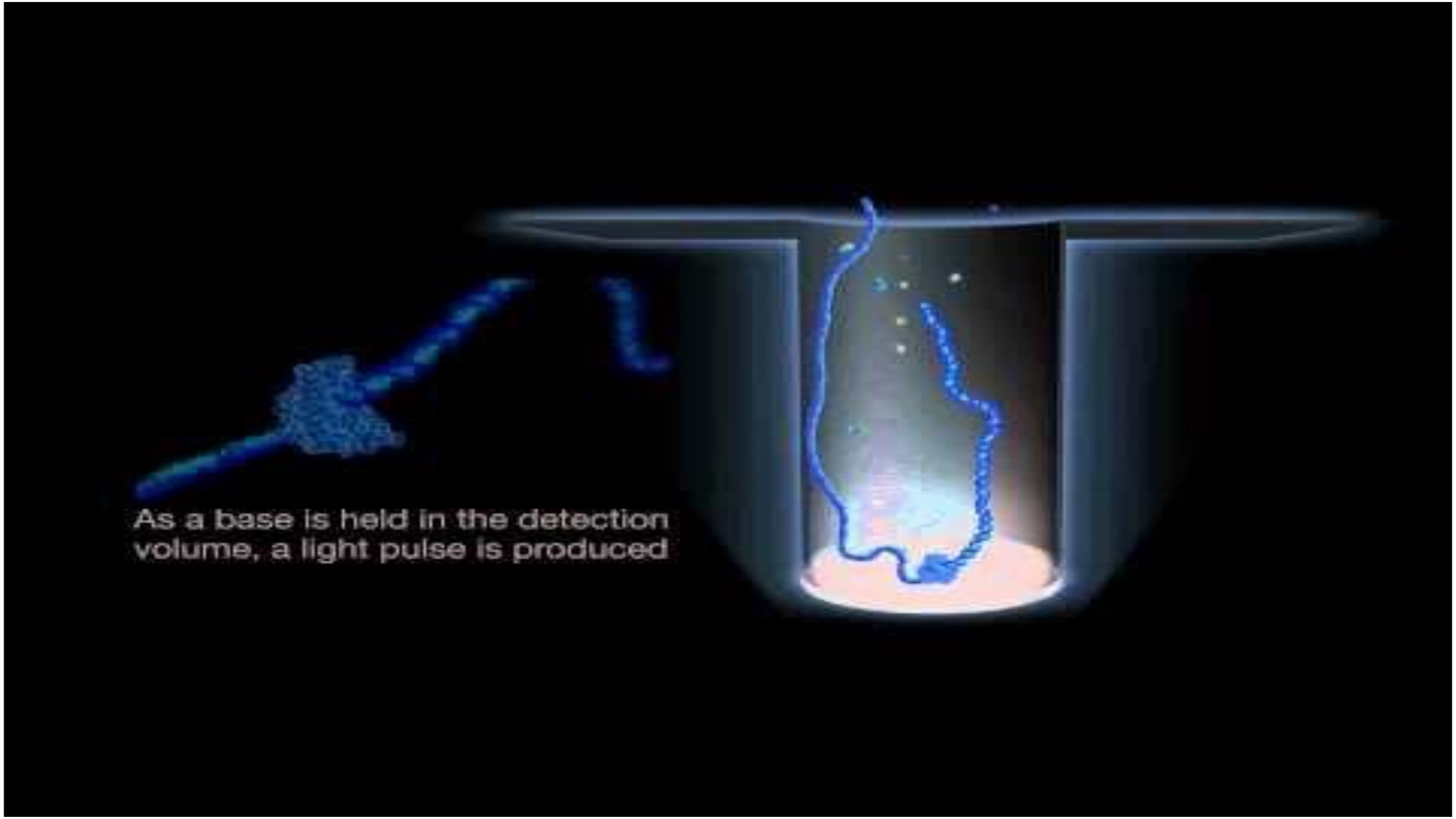
- The principle behind SMRT Sequencing involves the use of zero-mode waveguides (ZMWs), which are tiny wells with dimensions smaller than the wavelength of visible light. These ZMWs allow the observation of individual DNA polymerase molecules as they synthesize new DNA strands.



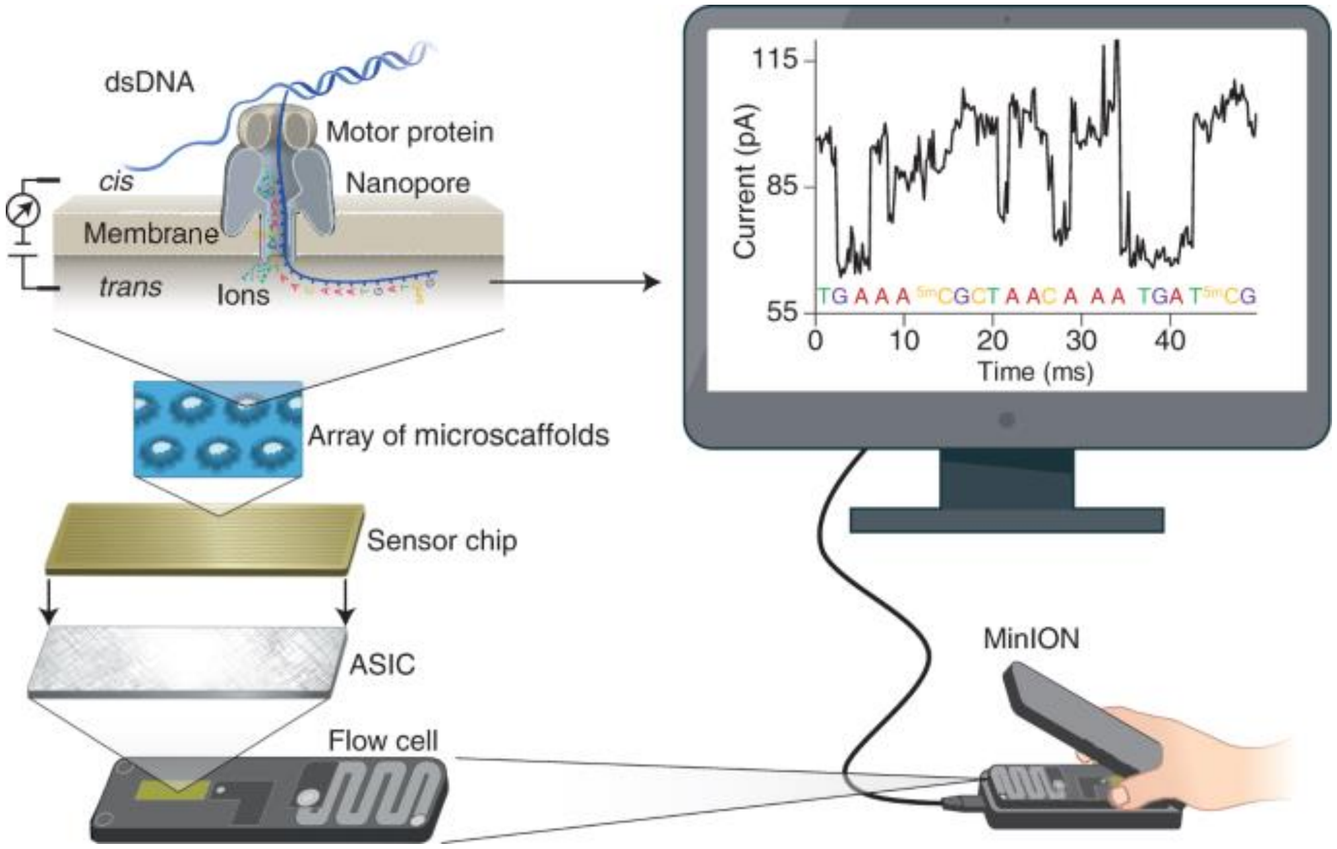
SMRT technology

source: Researchgate

- SMRT chip or cell (aluminium) contains approx. 10,000 ZMW's.
- ZMW is a Nano well with a diameter of 70 mm and 100 nm of depth & volume of 20 zeptolitres.
- Illuminated from below (Nanophotonic Confinement Structure).
- Single DNA Polymerase.
- Genomic DNA is fragmented & denatured.
- Each ssDNA ~10,000 nu
- Know Oligonucleotide is attached & a primer complementary to it is introduced.
- Allowed to diffuse on the CHIP.
- One ssDNA in one well.
- dNTP's are allowed to diffuse (dATP(**BLUE**), dGTP(**GREEN**), dTTP(**YELLOW**), dCTP(**RED**)
- Single nucleotides are incorporated at a time, a fluorescent tag is cleaved off & fluorescence is detected by a highly sensitive detector..
- Real-Time.
- Tiny detection volume provides a 1000-fold improvement in the reduction of background noise.


As a base is held in the detection volume, a light pulse is produced

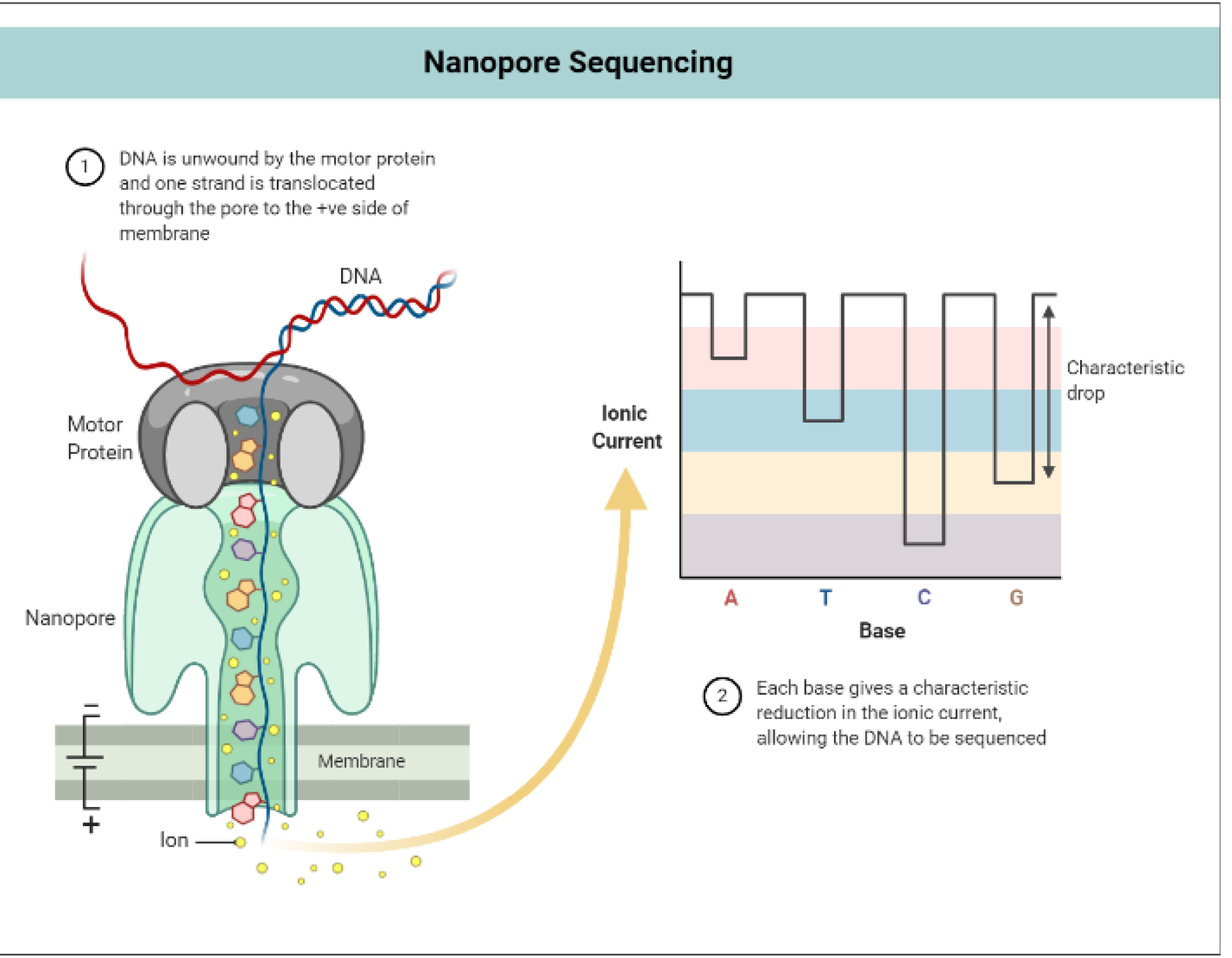
Nanopore Sequencing

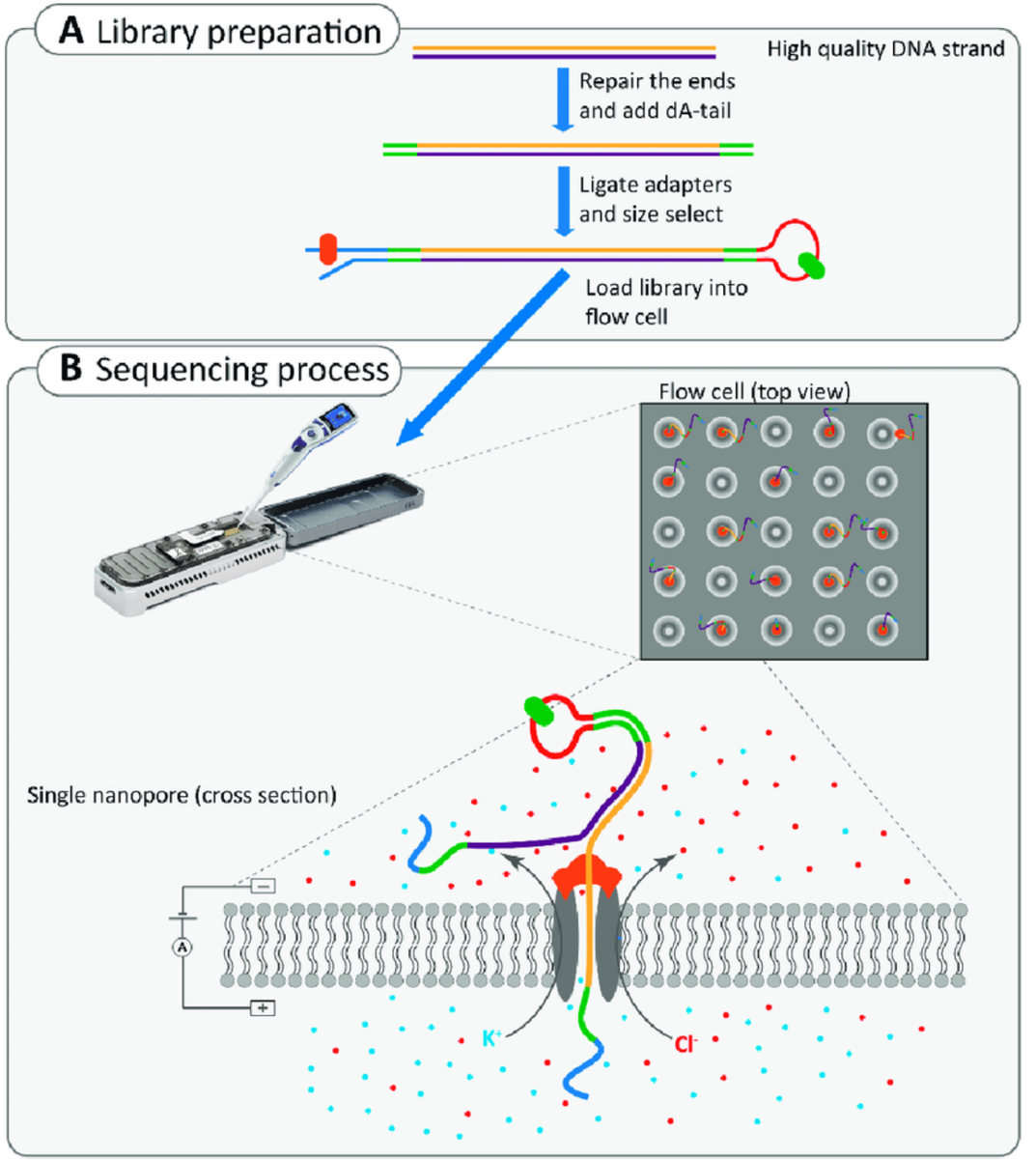


Nanopore Sequencing
source: Researchgate

- It’s ability to sequence long DNA fragments in real-time without the need for amplification or labeling. This technology, pioneered by Oxford Nanopore Technologies, offers a unique approach to DNA sequencing by monitoring changes in electrical current as DNA molecules pass through nanoscale pores.

- The principle behind Nanopore Sequencing relies on the detection of characteristic disruptions in the ionic current as DNA strands are threaded through a protein nanopore embedded in an electrically resistant membrane.





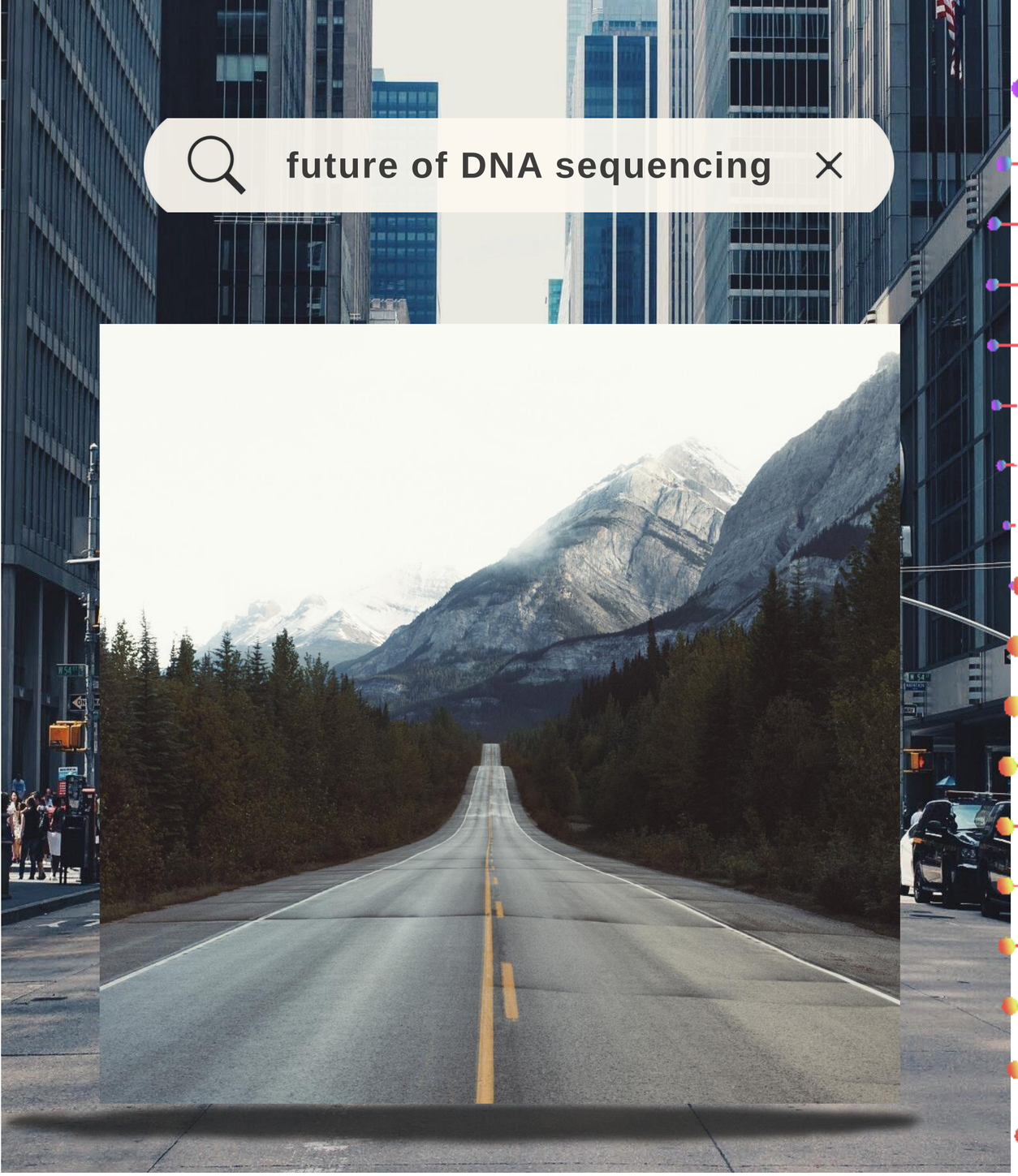
Nanopore Sequencing

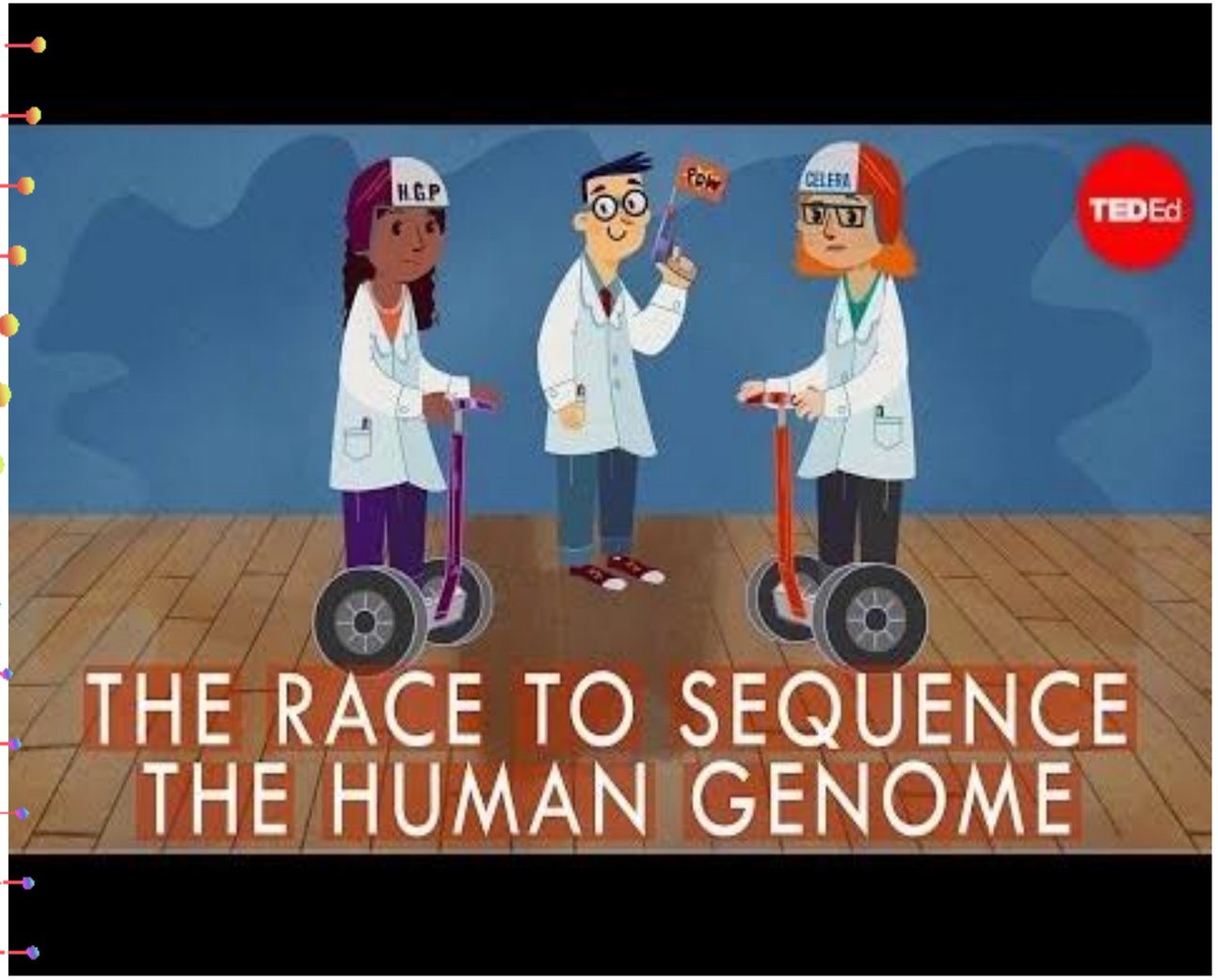
source: Researchgate


is a straightforward process,

- Nanopore Sequencing offers several unique advantages, including:
- Long Read Lengths
- Real-Time Sequencing
- Portability
- Direct Detection of Base Modifications
- While nanopore sequencing initially had lower accuracy compared to other methods, continuous improvements in pore engineering, base-calling algorithms, and sample preparation techniques have significantly enhanced its performance. Nanopore Sequencing has found applications in areas such as de novo genome assembly, structural variation analysis, transcriptomics, and metagenomics, among others.

Method	Accuracy	Applications
Sequencing by Synthesis	High	High throughput, cost-effective
Ion Semiconductor Sequencing	High	Fast, scalable
SMRT Sequencing	Very High	Epigenetic studies, long reads
Nanopore Sequencing	Variable	Real-time, field applications


future of DNA sequencing


H.G.P
CELERA
TED-Ed
THE RACE TO SEQUENCE
THE HUMAN GENOME

References

SBS Illumina (Sequencing by Synthesis):

- Mardis, E. R. (2008). Next-generation DNA sequencing methods. Annual Review of Genomics and Human Genetics, 9, 387-402. https://doi.org/10.1146/annurev.genom.9.081307.164359
- Bentley, D. R., Balasubramanian, S., Swerdlow, H. P., Smith, G. P., Milton, J., Brown, C. G., ... & Smith, A. J. (2008). Accurate whole human genome sequencing using reversible terminator chemistry. Nature, 456(7218), 53-59. https://doi.org/10.1038/nature07517

Ion Semiconductor Sequencing:

- Rothberg, J. M., Hinz, W., Rearick, T. M., Schultz, J., Mileski, W., Davey, M., ... & Bustillo, J. (2011). An integrated semiconductor device enabling non-optical genome sequencing. Nature, 475(7356), 348-352. https://doi.org/10.1038/nature10242
- Rhoads, A., & Au, K. F. (2015). PacBio sequencing and its applications. Genomics, Proteomics & Bioinformatics, 13(5), 278-289. https://doi.org/10.1016/j.gpb.2015.08.002

SMRT Sequencing (Single Molecule Real-Time Sequencing):

- Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., ... & Turner, S. (2009). Real-time DNA sequencing from single polymerase molecules. Science, 323(5910), 133-138. https://doi.org/10.1126/science.1162986
- Rhoads, A., & Au, K. F. (2015). PacBio sequencing and its applications. Genomics, Proteomics & Bioinformatics, 13(5), 278-289. https://doi.org/10.1016/j.gpb.2015.08.002

Nanopore Sequencing:

- Deamer, D., Akeson, M., & Branton, D. (2016). Three decades of nanopore sequencing. Nature Biotechnology, 34(5), 518-524. https://doi.org/10.1038/nbt.3423
- Jain, M., Olsen, H. E., Paten, B., & Akeson, M. (2016). The Oxford Nanopore MinION: Delivery of nanopore sequencing to the genomics community. Genome Biology, 17(1), 239. https://doi.org/10.1186/s13059-016-1103-0

Future of DNA Sequencing:

- Goodwin, S., McPherson, J. D., & McCombie, W. R. (2016). Coming of age: Ten years of next-generation sequencing technologies. Nature Reviews Genetics, 17(6), 333-351. https://doi.org/10.1038/nrg.2016.49
- Shendure, J., Balasubramanian, S., Church, G. M., Gilbert, W., Rogers, J., Schloss, J. A., & Waterston, R. H. (2017). DNA sequencing at 40: Past, present and future. Nature, 550(7676), 345-353. https://doi.org/10.1038/nature24286

Thank You...